

Effects of vineyard ‘potential’ and grape maturation on the aroma-volatile profile of Grenache wines

Ignacio Arias¹, Blanca Lacau², Jesús Astrain², Cristina Barón², Purificación Fernández-Zurbano³, Vicente Ferreira¹ and Ana Escudero^{1*}

¹Laboratoriode Análisis del Aroma y Enología (LAAE), Department of Analytical Chemistry, Universidad de Zaragoza, Instituto Agroalimentario de Aragón (IA2) (UNIZAR-CITA), Associate Unit to Instituto de Ciencias de la Vid y del Vino (ICVV) (Universidad de La Rioja–CSIC–Gobierno de La Rioja), calle Pedro Cerbuna 12, 50009 Zaragoza, Spain

²Bodegas Pirineos, S. A. Carretera Barbastro-Naval km 3.5, 22300 Barbastro, Huesca, Spain

³Instituto de Ciencias de la Vid y el Vino (ICVV) (Universidad de La Rioja–CSIC–Gobierno de La Rioja), Carretera de Burgos km 6, Finca La Grajera, 26007 Logroño, La Rioja, Spain

*Corresponding author: escudero@unizar.es

ABSTRACT

Background and aims: Wine is a beverage characterized by its pleasant aromatic features. These sensory notes are determined by the specific concentrations of odorous chemical compounds in each wine. Many of these aroma compounds arise directly or indirectly from the grapes, and their formation is affected by both grape metabolism and the viticultural ecosystem. Two studies were done with the 2015 vintage of *Vitis vinifera* L. cv. Grenache in Denominación Origen Somontano, a wine region of northern Aragon, Spain. In one study, we analysed wine from vineyards with different potentials: high potential, defined by balanced yield and high exposed leaf area (*surface foliaire exposée*, SFE, expressed in square metres) relative to production (P, expressed as kilograms of grapes) (i.e. high SFE:P ratio); and low potential, defined by unbalanced yield and low SFE:P ratio. In the other study, we analysed wine produced from grapes harvested at different times and therefore at different stages of ripening. The aim was to determine the effects of these variables on the aromatic compound profile of the wines.

Methods and results: Concentrations of major aroma compounds were determined by gas chromatography (GC) with flame ionization detection, those of minor and trace aroma compounds by GC–mass spectrometry (MS), and those of pyrazines by thermal desorption–GC coupled with GC–MS. In the first study, wines from high-potential vineyards had higher concentrations of some compounds, such as esters of fermentative origin (isoamyl acetate, ethyl lactate and diethyl succinate), esters of varietal origin (ethyl dihydrocinnamate, methyl vanillate and ethyl vanillate), and terpenols (linalool and geraniol). In contrast, wines from low-potential vineyards had higher concentrations of 3-isopropyl-2-methoxypyrazine, γ -nonalactone and volatile phenols. In the second study, concentrations of varietal compounds such as rotundone and linalool increased with extended maturation. Furthermore, as ripening progressed, the ester to acid ratio for linear fatty acid ethyl esters generally increased while that for branched fatty acid ethyl esters tended to decrease. Acetaldehyde concentration was decreased in wines produced from grapes harvested at the latest date, a result that may be related to increased polyphenol content.

Conclusions: The results of these studies provide an approximation of how the aromatic compound profile of a Grenache wine may differ between vineyards with different characteristics.

Significance and impact of the study: We suggest explanations for these differences, which may guide the choice of harvest date.

KEYWORDS

Grape maturation, vineyard selection, wine aroma

INTRODUCTION

A large number of factors must be controlled and a great deal of information considered to create a high-quality product with specific characteristics and properties. This is especially true for wine, which is a beverage of enormous complexity (De-La-Fuente-Blanco *et al.*, 2017). Within the matrix of elements conferring this complexity, aroma plays a key role in the perception of the quality and characteristics of the wine (Ferreira *et al.*, 2009). Therefore, it is essential to understand which factors are most important in determining a wine's aromatic compound profile.

These factors depend largely on the variety of the grape from which the wine is produced. Grenache is one of the most widely grown *Vitis vinifera* varieties in the world, especially in Spain, where it is used to produce many wines with appellations of origin, such as Rioja and Somontano. Wines produced from Grenache are neutral and have a high alcohol content, therefore the vineyard must be thoroughly controlled to obtain the best results. This makes it an interesting variety to study, because the world is experiencing a series of environmental shifts that may be changing our understanding or attitudes towards vineyards and winemaking. The clearest example of these shifts is the increase in the average temperature of the planet's surface, which in turn means an increase in the alcohol content of wines, as well as expedited grape maturation and earlier harvest (Edwards *et al.*, 2017).

Against this background, and given that the market does not currently favour full-bodied wines with high alcohol content, many wineries have opted to harvest slightly earlier. However, this practice can have consequences that winemakers and other wine professionals cannot control (Jones and Davis, 2000). Therefore, we need greater knowledge within the field of viticultural research to develop the tools and techniques necessary to manage the effects of climate change on the wine industry in a consistent manner. However, few detailed studies have been carried out on how different vineyard characteristics and different degrees of grape maturation affect a wine's flavor and aroma profile.

Grape variety, climatology, soil and production system influence grapevine vigour and thus determine the characteristics of the canopy,

microclimate, health and maturation of the grape; all these factors determine the final quality of the wine (Reynolds and Heuvel, 2009). Therefore, an understanding of these vineyard characteristics is fundamental if we are to propose management techniques that ensure optimal grape conditions at harvest and consequently the production of high-quality wine (Marcon *et al.*, 2015). At present, exposed leaf area (*surface foliaire exposée*, SFE, expressed in square metres) relative to production (P, expressed as kilograms of grapes), that is, SFE:P ratio, is one of the indicators most commonly used to control grape quality. For single canopy-type trellis-training systems, the SFE:P ratio required to maximize total soluble solid content, berry weight and berry colour at harvest ranges from 0.8 to 1.2 m²/kg (Kliewer and Dokoozlian, 2005).

Another important factor in the vineyard environment is grape maturity. The ripening process is extremely complex, with concentrations of precursors and metabolites increasing or decreasing significantly over time (Coombe and McCarthy, 1997). Harvesting on one day as opposed to another can result in significant differences in the end product, that is, the wine. However, our understanding of how changes during ripening influence the aromatic compound profile of a wine remains limited. Currently, wineries usually evaluate sugar content, pH and acidity, and do colorimetric tests to evaluate the maturity of a vintage, but none of these variables indicate the aromatic potential of the grape (Yuan and Qian, 2016). Therefore, it would be useful to correlate some of these variables with the aromatic potential of a vintage.

To this end, we carried out two studies to determine the effects of varying two winemaking variables, that is, vineyard potential and grape maturity (in other words, the degree of ripeness of the grape), on the aroma compound profile of the wine end-product.

MATERIALS AND METHODS

1. Reagents, solvents and standards

High-performance liquid chromatography–quality dichloromethane, ethanol and methanol were supplied by Fisher Scientific (Loughborough, UK). LiChrolut EN resins were supplied by Merck (Darmstadt, Germany). Pure water was obtained from a Milli-Q purification

system from Millipore (Bedford, Germany). The standards (purity > 98% in all cases) were supplied by Merck, ChemService (West Chester, PA, USA), PolyScience (Niles, IL, USA), Lancaster (Eastgate, UK), Alfa Aesar (Karlsruhe, Germany), PanReac (Barcelona, Spain), Firmenich (Geneva, Switzerland), AromaLab (Planegg, Germany) and Oxford Chemicals (Hartlepool, UK).

2. Preparation of wine samples

2.1. Vineyard potential study

The first study focused on two different kinds of vineyard: high maturation potential and low maturation potential. Wines were prepared from grapes of the 2015 vintage of the Pirineos Winery, with the Somontano appellation of origin. Samples of each wine were then analysed in September 2016.

Five full-production and commercial vineyards were selected: three high-potential vineyards and two low-potential vineyards. The *Vitis vinifera* grapevine was cv. Grenache, grafted on a 110-Richter rootstock. Vineyard age ranged between 11 and 32 years. The vineyards are close to each other and at a similar altitude (443.4 ± 46.2 m). The soils are calcareous and poor in organic matter content.

The vineyard blocks (high and low potential) with a priori maximal diversity in quality were selected based on historical data and criteria derived from the commercial system Dyostem® (Vivelys, Villeneuve-lès-Maguelone, France). According to the manufacturer's instructions, this tool monitors sugar loading and changes in the colour of the fruit to classify grape quality and determine the optimal harvest date.

Numerous characteristics of the vineyard, as summarized in Table 1, were also considered.

The high-potential vineyards contained a balanced crop, which was always under good physiological and phytosanitary conditions, and their clusters were loose and homogeneous. Their mean yield was 5051 ± 1098 kg/ha and their mean SFE:P was 1.07 ± 0.15 m²/kg. In contrast, in the low-potential vineyards the strains showed an excess of vigour. Additionally, the bunches were more heterogeneous; some were loose with small berries, whereas others were compact with relatively swollen grapes. The mean yield of these vineyards,

8607 ± 523 kg/ha, was higher than that of the high-potential vineyards, but their mean SFE:P ratio, 0.6 ± 0.01 m²/kg, was lower; thus, the results show a patent imbalance.

Grapes were sampled at random from 15-kg boxes of grapes collected by hand, with the sampling adapted to the geography of the vineyard and the practicalities of the winery. Moreover, whenever possible, grapes from vines growing at the borderlines of the vineyard were not harvested.

Two kinds of vinification were carried out to obtain a more realistic view of the process. Grapes from two high-potential vineyards and one low-potential vineyard were processed using microvinification methods (i.e. microfermentation). Grapes from the other two vineyards (one high potential and one low potential) were processed using vinification methods used in industrial wineries.

Microvinification was carried out in 70-L tanks containing 40 kg of grapes. Processing was carried out in triplicate for grapes from each vineyard. First, the grapes were destemmed and squeezed lightly. To the resulting paste was added 0.05 g/L of SO₂ (in the form of metabisulfite; dilution, 1:10), dry ice and a dose of 0.008 mL/L pectolytic enzymes (Endozym ICS 10 Rouge, AEB, Stuttgart, Germany). Twenty-four hours later, all tanks were inoculated with Lalvin ICVD 254 (Lallemand, Montreal, Canada) at 10⁶ cells/mL, which was reactivated with the organic (85%) and inorganic (15%) nutrient mixture (0.2 g/L) INI-LEV (LEV2050, Alzoaín, Spain). Once alcoholic fermentation was complete, the wines were inoculated with the malolactic bacterium (*Oenococcus oeni*) strain Lalvin VP41 (Lallemand). After malolactic fermentation, free sulphur dioxide was adjusted to 30 mg/L. Then the wine was filtered and bottled.

Industrial vinifications carried out the fermentation in tanks of 10,000 L. Apart from this difference, the process and dosages of additives were the same as described previously for microvinification. A series of treatments were carried out for separate study to investigate the effects of ageing the wines after malolactic fermentation by means of the use of different doses of micro-oxygenation and by maceration using chips at different toasted degree.

TABLE 1. Characteristics of the vineyards selected for the studies.

Character	Vineyard ^a				
	High 1	High 2	High 3	Low 1	Low 2
Vineyard conditions					
Soil type	Fine sandy loam	Fine sandy loam	Fine sandy loam	Loam	Fine sandy loam
Altitude (m)	463	398	428	514	414
Row orientation	S–W	S–W	W	S–W	N–E
Vineyard age (years)	32	24	16	18	11
Vine spacing, maximum (m)	3.1 ´ 1.2	3.1 ´ 1.2	3.1 ´ 1.2	3.1 ´ 1.2	3.3 ´ 1.2
No. of vines/ha	2444	2366	2366	2444	2222
Yield and vegetative growth					
Mean no. of bunches in 10 vines	11.8	9.8	12.6	20.3	20.4
Mean bunch weight (kg)	0.208	0.166	0.178	0.166	0.198
Yield (kg/ha)	6000	3848	5306	8237	8976
Mean canopy width (m)	0.3	0.4	0.5	0.35	0.6
Mean canopy height (m)	1.1	0.8	0.8	0.8	1
Solar exposure (%)	50	40	60	50	50
Mean exposed leaf area (m ² /vine)	2.64	1.92	1.92	1.92	2.40
Mean SFE:P ratio	1.1	1.2	0.90	0.6	0.59
Agricultural practices					
Shoot thinning	Yes	Yes	Yes	Yes	No
Shoot tipping	Yes	Yes	Yes	Yes	Yes
Desuckering	Yes	Yes	Yes	Yes	No
Irrigation	No	No	No	No	No
Trellising	Double guyot	Double guyot	Double guyot	Double guyot	Double guyot
Mulching	No	No	No	No	No
Leaf plucking	No	No	No	No	No
Tilling	Yes	Yes	Yes	Yes	Yes
Vine and cluster status					
Leaf layers	3 or 4	3 or 4	3 or 4	3 or 4	> 4
Damaged leaves (%)	< 2	2–10	< 2	< 2	< 2
Water availability	Light stress	Medium	Medium	Light stress	Light stress
Growth cessation	Partial	Partial	Partial	Total	Null
Vigour	Moderate	Moderate	Moderate	Moderate	High
Affected cluster (%)	< 1	< 1	< 1	< 1	< 1
Cluster compactness	Medium	Small	Small	Medium	Big
Berry size	Small	Medium	Small	Small	Medium

SFE:P ratio, exposed leaf area (surface foliaire exposée, SFE, expressed in square metres) relative to production (P, expressed as kilograms of grapes).

^a‘High’ and ‘Low’ refer to the vineyard’s potential.

2.2. Grape maturation study

In the second study, grapes from one high-potential vineyard were harvested at three different times, calculated from *véraison*. At the earliest harvest time, 38 days post *véraison*, grapes were collected before they had reached optimal maturity; these were used to produce

wines 1–3 (mean pH, 3.29; mean alcohol content, 14.7% v/v). The second collection was 1 week later, 45 days after *véraison*; the harvested grapes were used to produce wines 7–9 (mean pH, 3.36; mean alcohol content, 15.3% v/v). The third collection was 3 weeks after the first, at 59 days post *véraison*; the harvested grapes were used to produce wines

13–15 (mean pH, 3.40; mean alcohol content, 17.8% v/v).

The wines were produced as described previously for microvinification in the first study, again with processing carried out in triplicate.

Details of each of the wines produced are summarized in Table 2.

3. Methods

3.1. Oenological variables

The following oenological variables of the 25 wines were determined: pH, volatile acidity, total acidity, residual sugar content, malic acid content, lactic acid content, ethanol content, easily assimilated nitrogen, colour index and total polyphenols index (TPI). All were evaluated according to the methods of the Office International de la Vigne et du Vin (OIV, 2018).

3.2. Quantitative analysis of methoxypyrazinas and rotundone

The alkylmethoxypyrazine and rotundone concentrations of the wines were determined using stir-bar sorptive extraction, followed by

thermal desorption gas chromatography coupled with mass spectrometry, using a procedure described previously (Wen *et al.*, 2018). A total of 5 mL of sample was transferred into a clean 25-mL Erlenmeyer flask, then 1 mL of 0.5 M citric acid–sodium citrate buffer was added to the same flask to adjust the pH to 5.4. After extraction (750 rpm for 30 min), the stir bar was desorbed using a thermal desorption unit and a cryocooled injection system (CIS 4) with a programmable temperature vaporization inlet equipped with an MPS autosampler (Gerstel, Müllheim an der Ruhr, Germany). The stir bar was thermally desorbed in the thermal desorption unit (splitless mode).

The analysis was carried out using an Agilent 7890A gas chromatograph equipped with a Deans switch device (Agilent Technologies, Santa Clara, USA), enabling the selective transfer of heart cuts from the first column to the second. The first column was a DB-5MS column (length, 15 m; internal diameter, 250 µm; film thickness, 0.25 µm) (J & W Scientific, Folsom, CA, USA) combined with a flame ionization detector and the Deans' switch. The second column was a Sapiens-WAX mass spectrometer (Teknokroma, Barcelona, Spain) (length, 30 m;

TABLE 2. Wines analysed in the vineyard potential and grape maturation studies

Wine number(s)	Vinification ^a	Vineyard potential ^b	No. of days post <i>véraison</i>	Wine sample code(s) ^a
1–3	Microvinification	High 1	38	HP1-D38-M1, HP1-D38-M2, HP1-D38-M3
4–6	Microvinification	High 2	45	HP2-D45-M1, HP2-D45-M2, HP2-D45-M3
7–9	Microvinification	High 1	45	HP1-D45-M1, HP1-D45-M2, HP1-D45-M3
10–12	Microvinification	Low 1	45	LP1-D45-M1, LP1-D45-M2, LP1-D45-M3
13–15	Microvinification	High 1	59	HP1-D59-M1, HP1-D59-M2, HP1-D59-M3
16	Industrial T0	High 3	45	HP3-D45-T0
17	Industrial T1	High 3	45	HP3-D45-T1
18	Industrial T2	High 3	45	HP3-D45-T2
19	Industrial T3	High 3	45	HP3-D45-T3
20	Industrial T4	High 3	45	HP3-D45-T4
21	Industrial T0	Low 2	45	LP2-D45-T0
22	Industrial T1	Low 2	45	LP2-D45-T1
23	Industrial T2	Low 2	45	LP2-D45-T2
24	Industrial T3	Low 2	45	LP2-D45-T3
25	Industrial T4	Low 2	45	LP2-D45-T4

Wines 1–15 were produced by microvinification of grapes from different vineyards and harvested at different times, and wines 16–25 by industrial vinification from grapes from different vineyards but harvested at the same time

The codes indicate the following: D38, D45 and D59, grapes harvested at 38, 45 and 59 days post *véraison*, respectively; HP1, HP2 and HP3, from high-potential vineyards 1, 2 and 3, respectively; LP1, LP2 and LP3, from low-potential vineyards 1, 2 and

internal diameter, 250 μm ; film thickness, 1 μm) connected directly to an Agilent 5975C mass spectrometer. A quadrupole mass detector was operated in selected ion-monitoring mode with electron ionization.

Stable isotope dilution analysis was used to quantify compounds with selected mass fragments. The compounds were methoxypyrazines (3-isopropyl-2-methoxypyrazine, IPMP; 3-isobutyl-2-methoxypyrazine, IBMP; and 3-*sec*-butyl-2-methoxypyrazine, SBMP) and a sesquiterpene (rotundone).

3.3. Quantitative analysis of major compounds

Twenty-nine major compounds were isolated from the wines by liquid-liquid extraction and then analysed by gas chromatography with flame ionization detection, following the method described by Ortega *et al.* (2001). The analytes were:

- **carbonyl compounds:** carbonyl compounds of fermentative origin (acetoin and diacetyl) and oxidation-related carbonyl compounds (acetaldehyde)

- **esters:** linear fatty acid derivatives (ethyl propanoate, ethyl butyrate, ethyl hexanoate, ethyl octanoate and ethyl decanoate) and esters of fermentative origin (ethyl lactate, diethyl succinate, ethyl acetate, isoamyl acetate and hexyl acetate)

- **alcohols:** isobutanol, 1-butanol, isoamyl alcohol, methionol, benzylic alcohol and β -phenylethanol, and two C6 alcohols (1-hexanol and *cis*-3-hexenol)

- **acids:** linear fatty acids (acetic acid, butyric acid, hexanoic acid, octanoic acid and decanoic acid) and branched fatty acids (isobutyric acid and isovaleric acid)

a **lactone:** γ -butyrolactone.

Analyses were carried out using a GC-3800 from Varian (Walnut Creek, CA, USA) equipped with a flame ionization detector. The column used was a DB-WAX from J & W (length, 30 m; internal diameter, 0.32 mm; film thickness, 0.5 mm). The carrier gas was helium (flow rate, 2.2 mL/min). A total of 2 μL of the sample was injected in split mode (1:20). Analytes were referred to a selected internal standard (4-hydroxy-4-methyl-pentanone, 2-butanol, 4-methyl-2-pentanol, 2-octanol, heptanoic acid

or ethyl heptanoate), and the selected method for calibration was determination of relative response factors.

3.4. Quantitative analysis of minor and trace compounds

Forty-three minor and trace compounds were isolated by solid-phase extraction and then analysed by gas chromatography coupled with a mass spectrometry detection system, as explained by Lopez *et al.* (2002). The analytes were:

- **carbonyl compounds:** an oxidation-related carbonyl compound (benzaldehyde) and norisoprenoids (β -damascenone, α -ionone and β -ionone)

- **esters:** branched-acid derivatives (ethyl isobutyrate, ethyl 2-methylbutyrate and ethyl isovalerate), esters of varietal origin (methyl vanillate, ethyl vanillate, ethyl dihydrocinnamate and ethyl cinnamate), and esters of fermentative origin (isobutyl acetate, butyl acetate and phenylethyl acetate)

- **alcohols:** 1-penten-3-ol, C6 alcohols (*trans*-2-hexenol, *cis*-2-hexenol and *trans*-3-hexenol) and two C8 alcohols (1-octen-3-ol and *trans*-2-octen-1-ol)

- **terpenols:** linalool, linalool acetate, α -terpineol, β -citronellol and geraniol)

- **volatile phenols:** guaiacol, *o*-cresol, 4-ethylguaiacol, *m*-cresol, 4-propylguaiacol, eugenol, 4-ethylphenol, 4-vinylguaiacol, *trans*-isoeugenol, 2,6-dimethoxyphenol, 4-vinylphenol, 4-allyl-2,6-dimethoxyphenol, vanillin and acetovanillone

- **lactones:** *trans*-whiskylactone, *cis*-whiskylactone, γ -nonalactone and γ -decalactone.

A Varian 450 GC gas chromatograph fitted to a Saturn 2200 electronic impact ion trap mass spectrometer (also from Varian) was used. The column was a DB WAXetr from J & W (length, 60 m; internal diameter, 0.25 mm; film thickness, 0.5 mm). The carrier was helium (flow rate, 1 mL/min). A total of 2 μL of sample was injected. A mass range of 35–220 m/z was recorded. A selective m/z relation was used for each analyte, which was also referred to a selected internal standard (2-octanol, 3,4-dimethylphenol or 3-octanone), for quantification by response factor.

4. Data analysis

All the analytical data for both the vineyard maturation study and the grape maturation study were subjected to one-way ANOVA. A pair-wise comparison test (Fisher's test) was applied to detect significant differences (significance level 95%).

All analyses were carried out with XLSTAT, version 2015 (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

We evaluated the classic oenological variables of the 25 wines produced for the two studies: pH, volatile acidity, total acidity, residual sugar content, malic acid content, lactic acid content, ethanol content, easily assimilated nitrogen, colour index and TPI. The results for all these variables indicated that each vinification had proceeded correctly. The concentrations of the volatile compounds found in the wines are shown in Supplementary tables 1–7.

1. Vineyard potential study

The ANOVA results showed significant differences between wines from high- and low-potential vineyards in terms of the concentrations of 14 of the 74 aromatic compounds quantified in 19 wines. Data for these compounds were used in the principal components analysis (PCA).

The PCA scatterplot represents 70.4% of the variation (Figure 1). Data points corresponding to each set of microvinification triplicate samples are close together. Data points corresponding to wines produced by industrial vinification are also closely grouped, despite the wines having been subjected to different treatments; this indicates that source vineyard is more important than either of the treatments in determining the aromatic compound profile of the wine.

Data points for wines from high-potential vineyards are on the left side of the PCA scatterplot, and those for wines from low-potential vineyards are on the right side. Bearing in mind that wines 1–15 were produced by microvinification and wines 16–25 by industrial vinification, the closest groupings are, nevertheless, of data points for wines from vineyards sharing certain characteristics, and not of those for wines subjected to similar treatments.

The scatterplot shows that Grenache wines from high-potential vineyards are richer in the following compounds:

- esters of fermentative origin, namely isoamyl acetate, ethyl lactate and diethyl succinate

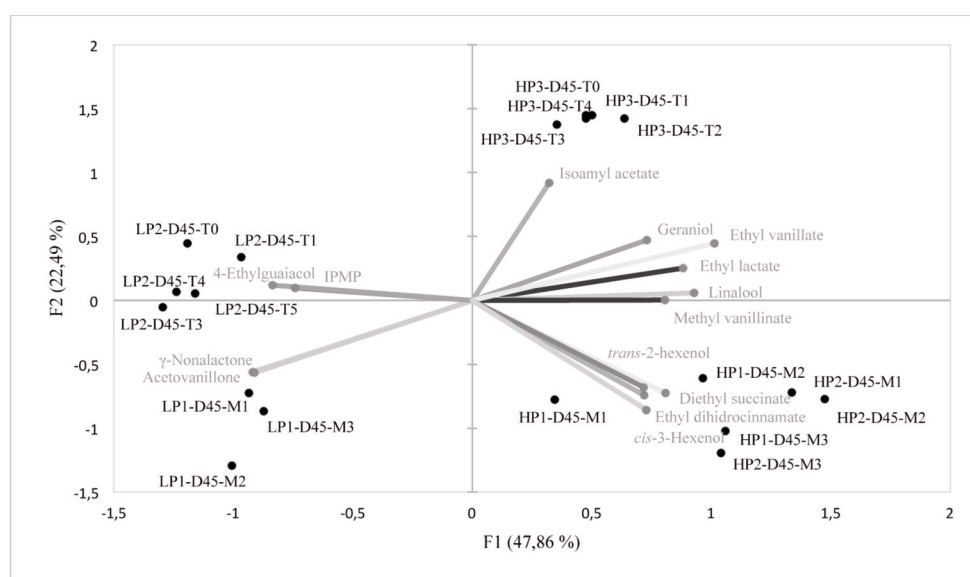


FIGURE 1. Vineyard potential study: principal components analysis scatterplot.

Projection on the first two principal components, using data for 14 aromatic compounds quantified in 19 samples of wine from low- and high-potential vineyards. Wine sample codes are defined in a footnote to Table 2.

- esters of varietal origin, namely ethyl dihydrocinnamate, methyl vanillate and ethyl vanillate

- terpenols, namely linalool and geraniol

- C6 alcohols, for example *trans*-2-hexenol and *cis*-3-hexenol.

In contrast, wines from low-potential vineyards are distinguished by higher concentrations of:

- volatile phenols, namely 4-ethylguaiacol and acetovanillone

- 3-isopropyl-2-methoxypyrazine

- γ -nonalactone.

Many of these results are due to the clear yield imbalance in low-potential vineyards, in which optimal grape maturation is not possible. The high rate of production in these vineyards means

TABLE 3. Wines analysed in the grape maturation study^a

Results for variables for which significant differences were found between samples of wines produced from grapes harvested 38, 45 and 59 days post *véraison*

Variable	38 days post <i>véraison</i> (<i>n</i> = 3)		45 days post <i>véraison</i> (<i>n</i> = 3)		59 days post <i>véraison</i> (<i>n</i> = 3)		<i>p</i>
	Mean	SD	Mean	SD	Mean	SD	
Classic variables							
pH	3.30 c	0.03	3.36 b	0.01	3.40 a	0.01	0.002
Volatile acidity (g/L)	0.21 b	0.25	0.39 b	0.11	0.75 a	0.06	0.02
Total acidity (g H ₂ T/L)	6.67 a	0.33	5.19 b	0.53	5.49 b	0.10	0.006
Malic acid (g/L)	0.72 a	0.14	0.65 ab	0.04	0.48 b	0.06	0.04
Ethanol (% v/v)	14.70 b	1.46	15.26 b	0.34	17.75 a	0.68	0.02
Esters							
Ethyl butyrate (mg/L)	0.25 a	0.10	0.23 a	0.04	0.07 b	0.01	0.02
Ethyl decanoate (mg/L)	0.03 b	0.00	0.06 ab	0.03	0.10 a	0.02	0.02
Butyl acetate (µg/L)	0.00 b	NA	3.04 b	1.47	4.89 a	0.45	0.002
Ethyl vanillate (µg/L)	267.07 a	34.73	171.48 b	17.86	280.11 a	48.72	0.02
Alcohols							
Isoamyl alcohol (mg/L)	259.87 b	5.70	314.16 a	17.77	256.90 b	12.48	0.003
Benzyl alcohol (mg/L)	0.86 b	0.35	2.24 a	0.37	2.83 a	0.47	0.002
β-Phenylethanol (mg/L)	29.04 b	3.27	32.97 b	3.29	39.48 a	0.66	0.009
<i>cis</i> -3-Hexenol (mg/L)	0.23 b	0.02	0.27 a	0.01	0.24 b	0.02	0.04
1-Octen-3-ol (µg/L)	17.61 a	5.80	21.93 a	5.95	3.81 b	1.29	0.009
<i>trans</i> -2-Octen-1-ol (µg/L)	3.47 a	0.94	2.72 a	0.59	ND b	NA	0.001
Acids							
Acetic acid (mg/L)	236.86 b	301.35	429.80 ab	171.14	795.14 a	22.56	0.04
Hexanoic acid (mg/L)	1.85 a	0.23	2.33 a	0.51	1.12 b	0.13	0.012
Terpenols							
Linalool (µg/L)	5.70 b	1.75	13.46 a	4.57	16.60 a	3.29	0.02
Linalool acetate (µg/L)	0.04 b	0.07	0.21 a	0.04	ND b	NA	0.004
Volatile phenols							
<i>o</i> -Cresol (µg/L)	2.70 a	0.21	1.71 b	0.80	ND c	NA	0.001
<i>m</i> -Cresol (µg/L)	ND b	NA	ND b	NA	0.51 a	0.10	< 0.0001
2,6-Dimethoxyphenol (µg/L)	58.94 c	19.03	90.38 b	17.84	132.93 a	5.00	0.003
Ethyl dihydrocinnamate (µg/L)	9.03 a	0.76	7.68 b	0.81	ND c	NA	< 0.0001
Lactone							
γ-Butyrolactone (mg/L)	22.84 b	2.65	32.06 a	4.91	23.39 b	1.51	0.03
Sesquiterpene							
Rotundone (ng/L)	0.02 b	0.01	0.11 ab	0.09	0.17 a	0.03	0.03

ND, not detected; NA, not available; SD, standard deviation.

^aData were subjected to ANOVA; the letters a, b and c indicate significant differences ($p \leq 0.05$) between wines produced from grapes harvested at the three different times.

that there is no opportunity for the vines to develop the metabolic routes of synthesis and degradation of the different aromatic compounds associated with normal ripening, as occurs in high-potential vines. This would explain the higher concentrations of varietal compounds such as terpenes (Friedel *et al.*, 2016) and fermentative compounds such as esters (Liu *et al.*, 2015; Bubola *et al.*, 2019), and the lower concentration of methoxypyrazines (de Boubée *et al.*, 2000; Mozzon *et al.*, 2016), in wines from high-potential vineyards.

Concentrations of *trans*-2-hexanol and *cis*-3-hexanol did not pass the olfaction threshold. Therefore, they do not have a significant effect on the aroma of Grenache wines from high-potential vineyards.

The higher concentrations of phenols and γ -nonalactone in wines from low-potential vineyards is surprising, because γ -nonalactone has been found in overripe grapes (Pons *et al.*, 2017; Allamy *et al.*, 2018). A possible explanation for the increased γ -nonalactone content is the lower leaf to crop ratio in low-potential vineyards, which makes the grapes more susceptible to sunburn and shrivelling. Of the phenols found, 4-ethylguaiaicol has a microbial origin and acetovanillone originates from the grape. Therefore, there seems to be no obvious causality between vineyard potential and

concentrations of members of this family of compounds.

It is worth highlighting the higher concentrations of esters of fermentative origin in wines produced by industrial vinification, as opposed to microvinification, using grapes from high-potential vineyards. This is the result of stricter conditions of anaerobiosis in the industrial vinification (Ferreira *et al.*, 1995).

2. Grape maturation study

Data for 85 variables for the nine samples of wine produced from grapes harvested at different times were subjected to ANOVA. Table 3 summarizes the results for the 25 variables for which significant differences were found.

There are clear differences between wines produced from grapes harvested at the three different times (see Table 3). Extended ripening time was associated with increased concentrations of some esters (ethyl decanoate, butyl acetate and ethyl vanillate), as has been described previously (Bindon *et al.*, 2013). The results for alcohols did not show a consistent pattern. Concentrations of linear fatty acids appear to have decreased with ripening. The concentration of acetic acid increased with ripening, as would be expected, because concentrations of sugars also increased.

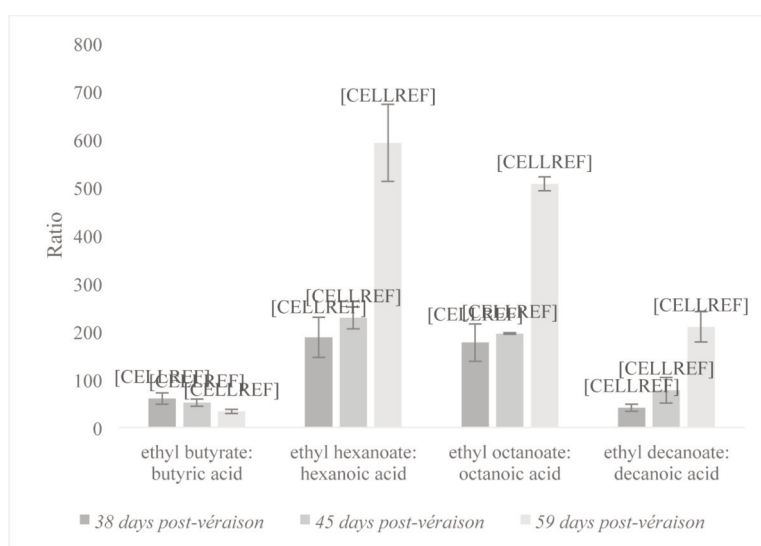


FIGURE 2. Ratios of esters derived from linear fatty acids to their corresponding acids, in samples of wines produced from grapes harvested at three different times.

Each ratio was calculated by dividing the concentration of the ester by the concentration of its corresponding acid and multiplying the result by 1000. Error bars were calculated as standard deviation/(3)^{1/2} (the denominator, 3, being the n number). Different letters indicate the existence of a significant difference between wines ($p < 0.05$; Fisher's post hoc test).

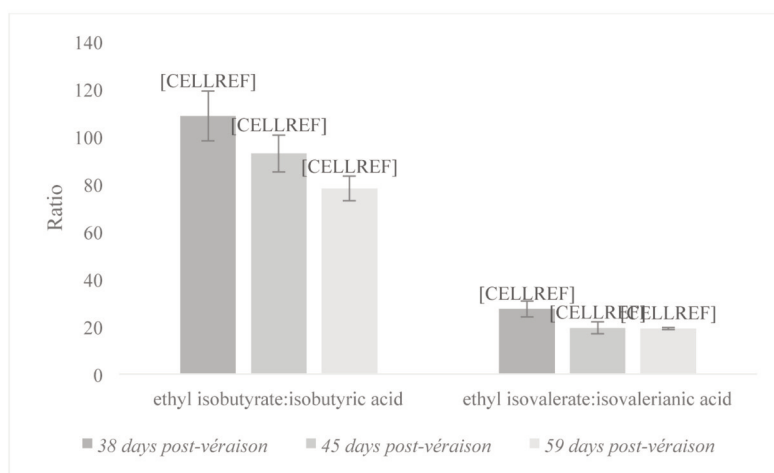


FIGURE 3. Ratios of esters derived from branched fatty acids to their corresponding acids, in samples of wines produced from grapes harvested at three different times.

Each ratio was calculated by dividing the concentration of the ester by the concentration of its corresponding acid and multiplying the result by 1000. Error bars were calculated as standard deviation/(3)^{1/2} (the denominator, 3, being the n number). Different letters indicate the existence of a significant difference between samples ($p < 0.05$; Fisher's post hoc test).

Concentrations of linalool and rotundone were increased in the wines produced from grapes harvested at later dates, consistent with the results of previous studies (Caputi *et al.*, 2011; Geffroy *et al.*, 2014; Marais, 2017).

It is important to point out the connection between harvest time and concentrations of esters and acids in the wine. Traditionally, varietal compounds such as rotundone and linalool have been considered important markers of grape maturity. However, in Grenache wines these compounds do not exceed the olfactory threshold, and therefore sensorial differences may be more clearly explained by variability in concentrations of important aromatic vectors such as esters and acids.

2.1. Ester to acid ratio

Given the complexity of the results, and the associated difficulty in interpreting them, we present a holistic view to aid understanding of the influence of harvest time on concentrations of the principal aromatic compounds.

We determined the ratio of esters derived from linear fatty acids to their corresponding acids for the wines produced from grapes harvested at the three different times. Figure 2 shows the ratios of esters and acids C4, C6, C8 and C10.

In the same way, we determined the ratio of esters derived from branched fatty acids to their corresponding acids for the wines produced from

grapes harvested at the three different times. Figure 3 shows the ratios for the esters ethyl isovalerate and ethyl isobutyrate.

As ripening proceeded, ratios of several esters derived from linear fatty acids to their corresponding linear fatty acids increased significantly, whereas ratios of esters derived from the equivalent branched fatty acids showed the inverse. Thus, in wines produced from grapes harvested early, we found a low proportion of linear esters relative to their acids, but this proportion increased as the grapes ripened. Conversely, the proportion of branched esters relative to their corresponding acids was higher at 38 days post *véraison* than at 45 and 59 days post *véraison*; however, these variations were significant for isobutyrate relative to isobutyric acid but not for isovalerate relative to isovalerianic (see Figure 3).

The volatile fatty acids in wine are produced during fermentation and by the action of certain microorganisms, yeasts and bacteria, by means of different, complex metabolic routes (Lambrechts and Pretorius, 2000). In contrast, ethyl esters are formed by reactions between ethanol and fatty acids or non-volatile organic acids (Etiévant, 1991), and this synthesis depends on multiple factors (Lambrechts and Pretorius, 2000). These esters may be present in grapes but are mainly produced during fermentation as a result of the secondary metabolism of yeasts, or even during ageing

(Etiévant, 1991). Over the lifetime of a wine, the linear ester content decreases and the branched ester content increases, with each kind of ester maintaining a constant hydrolytic equilibrium with its corresponding acid. Additionally, the balance between the enzymes that synthesize the esters helps determine the ratio of accumulated esters (Swiegers *et al.*, 2005).

Considering the information presented so far, it is difficult to provide a single explanation for variations in ester to acid ratios in wines produced from grapes harvested at different times. However, it is evident that ripening time influences the proportion of esters relative to the fatty acids from which they are derived.

Branched fatty acids, such as isobutyric (2-methylpropanoic), isovaleric (3-methylbutyric acid) and 2-methylbutyric, are by-products of protein metabolism by yeasts. In contrast, esters from branched fatty acids (ethyl 3-methylbutyrate, ethyl 2-methylbutyrate and ethyl isobutyrate) are mainly formed during ageing, although their production is also linked to the amino acid content of the grapes (Etiévant, 1991). Ratios of branched esters to their corresponding acids appeared to decrease as ripening progresses. However, to achieve a greater understanding of this phenomenon, it would be necessary to know the amino acid profile of the must at different harvest times.

2.2. Acetaldehyde concentration

Acetaldehyde concentration varied in wines produced from grapes harvested at different times (Figure 4). The difference was not significant, owing to the high variability in wines produced from grapes harvested at 45 days post *véraison*. However, acetaldehyde concentration in wines produced from grapes harvested at 59 days post *véraison* was significantly lower than in wines produced from grapes harvested at 38 days post *véraison* ($p = 0.0002$).

Total polyphenols index varied as ripening progressed, and was significantly higher in wines produced from grapes harvested at 59 days than in wines produced from grapes harvested at 38 days post *véraison* ($p = 0.001$) (see Figure 4).

We found a significant inverse correlation ($r = -0.77$, $P = 0.016$) between acetaldehyde concentration and TPI, which suggests an influence of polyphenol content on the concentration of free acetaldehyde, with consequences for the aroma of the wine. However, this influence would be expected to be limited, and it is difficult to explain how the increase in TPI would fully explain the decrease in acetaldehyde concentration (data found in other maturation experiments, data not shown). Other factors must be involved.

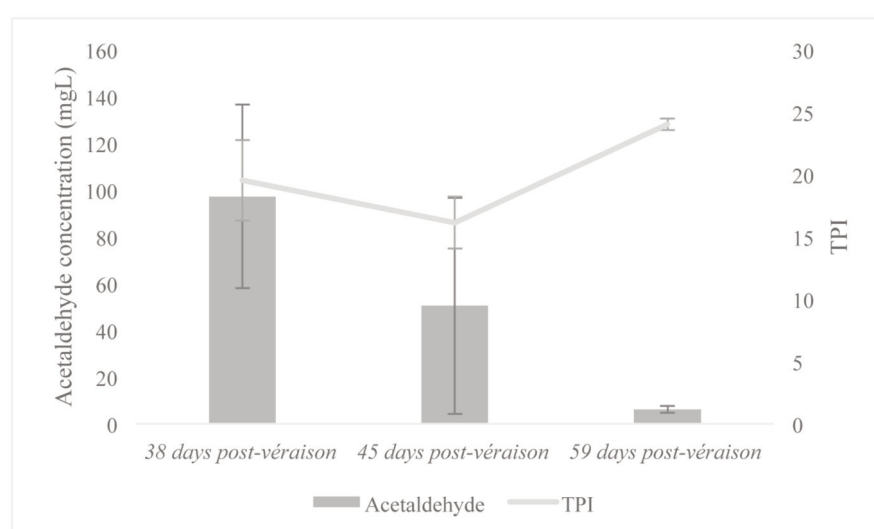


FIGURE 4. Acetaldehyde concentration and total polyphenols index (TPI) in wines produced from grapes harvested at three different times.

Error bars were calculated as standard deviation/ $(3)^{1/2}$ (the denominator, 3, being the n number).

According to Belda *et al.* (2017) and Bueno *et al.* (2018), acetaldehyde concentration in wine depends on three factors:

- sulphur dioxide content, given the strong adducts between sulphur dioxide and acetaldehyde
- polyphenol content
- yeast metabolism (because the characteristics of the medium in which the yeast grows, i.e. the composition of the must, differs according to harvest date).

The first of these factors can be ignored, because the same quantity of sulphur dioxide was added in each microvinification.

Polyphenols play a fundamental role in acetaldehyde content (Bueno *et al.*, 2018). It may be that ripening has an effect not so much on the polyphenols but on their ability to react with aldehydes.

Other factors that might also affect acetaldehyde concentration include factors that affect the yeast, such as stress or nutrient availability (Lambrechts and Pretorius, 2000). The yeast's ability to reduce aldehyde concentration may be limited in musts from unripe grapes, or the medium may be deficient in NADH or NADPH, which would have been consumed during more indispensable functions such as membrane formation. In such cases, the yeast would fail to reduce acetaldehyde to alcohol, which would account for the high concentration of the former.

CONCLUSIONS

The results of the present study suggest how vineyards with different characteristics can produce Grenache wines with distinct aromatic compound profiles. Wines from high-potential vineyards have higher concentrations of esters and terpenes, whereas those from low-potential vineyards have higher concentrations of some phenols and methoxypyrazines.

We have presented preliminary results related to the ripening process in the Grenache variety in one vintage, such as how the ratio of linear fatty acid ethyl esters to their corresponding acids is increased at the latest harvest date. Another finding is that acetaldehyde concentration is decreased in wines produced from the most mature grapes. This finding appears to be important, given the contribution of

acetaldehyde to a wine's sensorial character and its role in limiting the shelf life of wine.

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